PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Docket No: Q60438

Mitsuyuki MATSUMOTO, et al.

Appln. No.: 09/622,439

Group Art Unit: 1647

Confirmation No.: unknown

Examiner: S. WEGERT

Filed: August 17, 2000

For: POLYNUCLEOTIDES ENCODING SREB2 RECEPTOR

SUBMISSION OF EXECUTED DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
Washington, D.C. 20231

Sir:

Submitted herewith is an executed Declaration Under 37 C.F.R. §1.132 signed by Jun TAKASAKI.

Respectfully submitted,

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MATSUMOTO, M., *et al.*
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DECLARATION UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Jun Takasaki do declare and state:

THAT I am a citizen of Japan,

THAT I received a Bachelor's of Science in Applied Biological Science from Tokyo University of Science in March 1989, a Master of Science in Applied Biological Science from Tokyo University of Science in March 1991 and a Doctorate in Biological Science in 1999 from Tokyo University of Science;

THAT I have been employed by Yamanouchi Pharmaceutical Co., Ltd. since 1991 where I hold a position as Research Scientist in the Molecular Medicine Research Laboratories, Institute for Drug Discovery Research, at Yamanouchi Pharmaceutical Co., Ltd.

THAT I am a co-inventors of the inventions described in the specification of the above-identified application (hereinafter, referred to as the "present application")

THAT the following experimentation was carried out by myself, by the other co-inventors of the present application, or under their supervision.

EXPERIMENTATION

Methods

1. Animal and design of the experiment

1.1. Kainate-induced seizures

Male C57BL/6NCrj mice (5 week) were maintained on a 12-h light/dark cycle, with food and water available *ad libitum*. Mice received ~~intraperotoneal~~ intraperitoneal injections of kainic acid (30 mg/kg dissolved in saline, Sigma). Mice were sacrificed after the kainic acid (KA) injection on the following schedule: 0(n=5), 1(n=5), 2(n=5) and 7 days (n=5) -post-KA. Brains were rapidly removed, and hippocampi were dissected. J.T 5/22/02

1.2. Transgenic mice expressing human beta-amyloid precursor protein

Transgenic mice overexpressing the human βAPP with the "Swedish" mutation, Tg2576 (HuAPP695.K670N-M671L), were used in this experiment. The male transgenic mice were procured through Mayo Medical Ventures (USA) and crossbred with female B6SJLF1/J mice (Jackson Laboratory, USA) at Yamanouchi Pharmaceutical Co. Ltd (Japan). Only male mice of the offspring, that were housed individually and given food and water *ad libitum*, were analyzed. Three transgenic mice and non-transgenic mice at the age of 6 months were sacrificed. Brain were rapidly removed.

2. Quantitative analysis of SREB2 mRNA by reverse transcription-polymerase chain reaction (RT-PCR)

Tissue from each animal were weight and homogenized in the amount of ISOGEN indicated by the supplier (NIPPON GENE). Total RNA were then extracted according to the instruction manual (NIPPON GENE). Optical density (OD) measurements were taken at 260 and 280 nm in order to load accurate amounts of RNA from each ~~animals~~ animal and verify the purity of the extractions. Total RNA from ~~tissues~~ tissue of each animal were treated with RNase-free DNase I (Promega) to eliminate contaminating genomic DNA. For RT-PCR, first-strand cDNA were synthesized from DNase-treated total RNA by SuperScript™ II RT (Invitrogen) according to the instruction manual.

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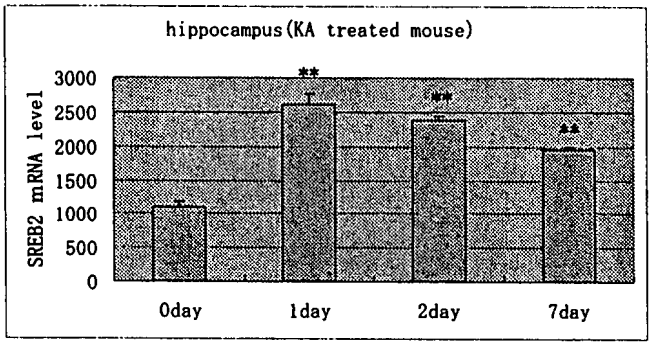
We quantified SREB2 mRNA by means of a Prism 7700 Sequence Detector (Applied Biosystems, Foster City, CA) with primer sets (5'-GACGTGGGCACTTACTCATTCAT-3' (SREB2-475F) and 5'-CTGAAGGAGCGGTGTTGGA-3' (SREB2-539R)).
RT-PCR was carried out in a 25 µl reaction mixture prepared with a TaqMan PCR core reagent (Applied Biosystems) containing an appropriately diluted cDNA solution, 0.2 µM of each primer. To obtain a calibration curve, we amplified a known amount of human genomic DNA using the same method as above. The number of mRNA copies per 1 µl of total RNA was calculated using the equation as described by Medhurst, A. D. et al (1). Statistical evaluation was done using Dunnett's test for comparisons with the control group **P<0.01 vs. control.

Results

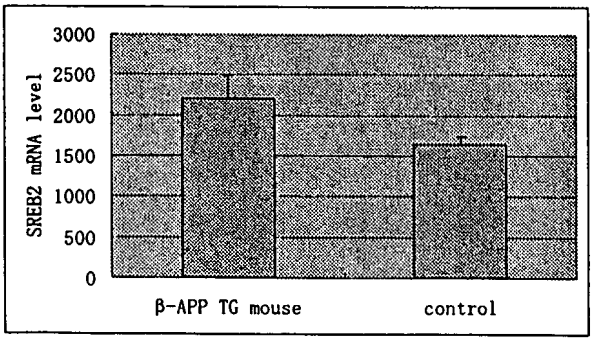
1. Kainate-induced seizures

As shown on Figure 1, the significant increase of SREB2 mRNA level were observed 1, 2 and 7 day after kainate injection.



2. transgenic mice expressing human beta-amyloid precursor protein

The level of expression of SREB2 in brain of transgenic mice expressing human β-APP were higher than that of normal mouse.



Discussions

1. Kainic acid-administered mouse

(1) The kainic acid (KA)-administered animal model is an animal model for the "temporal lobe epilepsy", in which systemic convulsion is observed for hours after the administration of KA and neurodegeneration in the hippocampus and subsequent abnormal sprouting of mossy fibers are observed, which causes neuronal plasticity and chronic spontaneous seizures (2-7).

With respect to this "temporal lobe epilepsy" animal model, it is known that neurodegeneration in the CA3 area, in which a large number of kainic acid receptors exists, occurs by the stimuli upon kainic acid receptors and that the neurodegeneration is observed at 24 hours following KA administration and reaches maximum within several days (3,5,7).

(2) As shown in the experimental results above, the expression level of SREB2 of the present invention was significantly increased, having the peak at one day after the KA administration, which corresponds to the timing when neurodegeneration is observed in the above-described "temporal lobe epilepsy" animal model.

In the response to the previous Office Action in the present application, it was explained that (i) SREB2 of the present invention is expressed in dentate gyrus neurons in the hippocampus (cf. LifeSpan BioSciences, Inc./Yamanouchi Isotopic *In Situ* Hybridization Study - MM1, March 13, 2002), and (ii) SREB2 of the present invention is a GPCR which has an activity to increase the transcription activity via SRE and CRE (cf. Example 6).

It is known that the stimulus which increases the transcription activity via SRE and CRE can be the stimulus that excites neurons (8,9). Accordingly, it is understood that SREB excites dentate gyrus neurons.

(3) It is believed that the excitation of the dentate gyrus neurons is potentiated after KA administration (i) because the expression level of SREB2 which has a neuron ~~exiting~~ exciting activity is increased in the hippocampus after KA administration and (ii) because the expression site of SREB2 is the dentate gyrus neurons in the hippocampus. J.T 5/22/02

It is believed that neurodegeneration in the CA3 area is enhanced because, as a result of potentiation of the excitation of the dentate gyrus neurons in the hippocampus, glutamic acid is over-released from the mossy fibers which are axons of dentate gyrus neurons (glutamic acid-containing excitatory neural pathway), and this hyperexcitability causes excitotoxicity to the mossy fiber-projecting CA3 area neurons (4,5,10).

(4) As described above, the experimental results shown above indicate that the overexpression of the SREB2 of the present invention causes neurodegeneration in the CA3 area neurons and worsen the "epilepsy" among the central nervous system diseases. Accordingly, the experimental results above clearly support that the SREB2 can be a target of the therapeutic agents for treating central nervous system diseases.

2. Transgenic mouse overexpressing human βAPP with the "Swedish" mutant, Tg2576 (HuAPP695.K670N-M671L)

(1) The APP-overexpressing mouse is an animal model for "Alzheimer's disease", which shows impairments in learning and memory from about 10 months of age. (11,12)

(2) As shown in the experimental results above, this animal model for "Alzheimer's disease" shows significant increase in the expression level of SREB2 according to the present invention at 6 months of age which is before showing the Alzheimer's disease-like symptoms.

(3) The hippocampus and the entorhinal cortex form a neural circuit (entorhinal cortex → granule cells of the dentate gyrus neurons in the hippocampus → CA3 area → schaffer recurrent collateral → CA1 area → entorhinal cortex). Various types of inputs for memory are transmitted from dentate gyrus granule cells to CA3 pyramidal cells via excitatory synapses where glutamic acid is a neurotransmitter (13). As explained in the response to the previous Office Action, the memory is circulation of signals in this circuit and it has been known that inhibition of neurotransmission in this circuit causes deterioration in the learning ability (14,15).

(4) As described in "1. Kainic acid-administered mouse", overexpression of SREB2 causes neurodegeneration in the CA3 area.

(5) Accordingly, it is considered that, in the above-described animal model for "Alzheimer's disease", the increase of the SREB2 expression causes neurodegeneration in the CA3 area and, as a result, shows Alzheimer's disease-like symptoms such as memory and learning impairments.

(6) As explained in the response to the previous Office Action, the specification of the present application describes that the present invention is to provide a GPCR protein as the target of therapeutic agents for central nervous system diseases (cf. page 7, lines 1-14 from the bottom) and substances capable of modifying the activity of GPCR can be therapeutic agents for the central nervous system diseases (cf. page 57, line 7 from the bottom to page 59 line 3 from the bottom) and thus it is apparent to one skilled in the art that the GPCR protein of the present invention can be a target of the therapeutic agents for treating central nervous system diseases relating to memory and learning.

The experimental results described above clearly show that the SREB2 of the present invention worsens the Alzheimer's disease-like symptoms such as memory and learning impairment. According, the experimental results above support that the SREB2 can be a target of the therapeutic agents for treating central nervous system diseases relating to memory and learning.

1. Medhurst, A. D., Harrison, D. C., Read, S. J., Campbell, C. A., Robbins, M. J., and Pangalos, M. N. (2000) *J. Neurosci. Methods* **98**, 9-20

2. Lothman EW, Collins RC. Kainic acid induced limbic seizures: metabolic, behavioral, electroencephalographic and neuropathological correlates. Brain Res. 1981; 218: 299-318.
3. Nadler JV.Minireview. Kainic acid as a tool for the study of temporal lobe epilepsy. Life Sci. 1981; 29: 2031-42.
4. Sloviter RS. Possible functional consequences of synaptic reorganization in the dentate gyrus of kainate-treated rats. Neurosci Lett. 1992; 137: 91-6.
5. Sperk G. Kainic acid seizures in the rat. Prog Neurobiol. 1994; 42: 1-32.
6. Ben-Ari Y. Cell death and synaptic reorganizations produced by seizures. Epilepsia. 2001; 42: 5-7.
7. Zagulska-Szymczak S, Filipkowski RK, Kaczmarek L. Kainate-induced genes in the hippocampus: lessons from expression patterns. Neurochem Int. 2001; 38: 485-501.
8. Davis S, Vanhoutte P, Pages C, Caboche J, Laroche S. The MAPK/ERK cascade targets both Elk-1 and cAMP response element-binding protein to control long-term potentiation-dependent gene expression in the dentate gyrus in vivo. J Neurosci. 2000; 20: 4563-72.
9. Schulz S, Siemer H, Krug M, Hollt V. Direct evidence for biphasic cAMP responsive element-binding protein phosphorylation during long-term potentiation in the rat dentate gyrus in vivo. J Neurosci. 1999; 19: 5683-92.
10. Aronica EM, Gorter JA, Paupard MC, Grooms SY, Bennett MV, Zukin RS. Status epilepticus-induced alterations in metabotropic glutamate receptor expression in young and adult rats. J Neurosci. 1997; 17: 8588-95
11. Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G. Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. Science. 1996; 274: 99-102.
12. Terai K, Iwai A, Kawabata S, Tasaki Y, Watanabe T, Miyata K, Yamaguchi T. beta-amyloid deposits in transgenic mice expressing human beta-amyloid

precursor protein have the same characteristics as those in Alzheimer's disease. Neuroscience. 2001; 104: 299-310.

13. The Neuron: Cell and Molecular Biology, Oxford University Press, New York, p395-423, 1991.
14. Sakimura K, Kutsuwada T, Ito I, Manabe T, Takayama C, Kushiya E, Yagi T, Aizawa S, Inoue Y, Sugiyama H, et al. Reduced hippocampal LTP and spatial learning in mice lacking NMDA receptor epsilon 1 subunit. Nature. 1995; 373: 151-155.
15. Tsien JZ, Huerta PT, Tonegawa S. The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. Cell. 1996; 87:1327-1338.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date : May 22, 2002 Name : Jun Takasaki

Jun Takasaki